INNOVATIVE METHODOLOGY

Charting the human respiratory tract with airborne nanoparticles: evaluation of the Airspace Dimension Assessment technique

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there are few methods to obtain information on the morphology of the distal lungs. Currently available techniques include computed tomography (CT) (11) and magnetic resonance imaging with hyperpolarized noble gases (27, 45), but these techniques are expensive, technologically demanding, and in the case of CT entail exposure to ionizing radiation (8). An alternative technique based on aerosol particle deposition in the airways, aerosol-derived airway morphometry (ADAM), has previously been described and has been shown to accurately assess airspace dimensions in the lungs (3, 10, 28, 30, 48, 50). This technique has also shown great promise in detecting alterations to lung morphology caused by medical interventions (19, 37, 38), by respiratory disease (4, 5, 18, 22, 39), or by alteration due to natural processes such as lung development and aging (48). However, the technique has not been adapted in clinical practice.

Typically, the different versions of ADAM use airborne particles in the size range 0.8–1 μm to maximize particle deposition by sedimentation and minimize influence of particle diffusion. However, particles in this size range also deposit by inertial impaction. As a consequence, a breathing procedure with low, controlled flow rates has to be used, and this can be challenging, especially for subjects with respiratory disease. Furthermore, this breathing procedure makes it demanding to measure at full inflation of the lungs, and therefore studies with ADAM in most cases need determination of lung static volumes.

An aerosol-based technique using nanoparticles (<100 nm), named Airspace Dimension Assessment (AiDA) (17, 23), has been shown to overcome these limitations and suggested to have additional advantages (17, 23). The most important suggested advantages are the following: 1) Nanoparticles allow for measurements with simpler breathing patterns with higher and less controlled flow rates (17). 2) A change in airspace radius has a larger effect on the measured particle half-life time for diffusion-controlled deposition than for sedimentation, and thus the sensitivity to changes in airspace radii is expected to increase (23). 3) Nanoparticles are assumed to have a greater ability to penetrate damaged and poorly ventilated airspaces (41) and thus to have better sensitivity pathological alterations in the lungs (23). 4) The mass concentration needed of inhaled nanoparticles is two to three orders of magnitude less than for micrometer-sized particles. 5) Finally, interpretation of data may also be simpler, as the deposition of nanoparticles is governed by diffusion only rather than a complex combination of sedimentation, diffusion, and inertial impaction (23).

INTRODUCTION

Injuries and changes in the smallest airspaces of the lungs are the first stage of many serious respiratory diseases. Yet
As described elsewhere, an instrument has been constructed and characterized for performing measurements of lung deposition of monodisperse nanoparticles from fixed volumetric sample depths during a breath hold (17). A theoretical model for using the data from this instrument to estimate effective airspace radii ($r_{\text{AiDA}}$) in the distal lungs has been suggested by Löndahl et al. (23). Clinical studies have shown that the deposition of nanoparticles is related to pulmonary function (1, 25, 33), lung density assessed by CT (1), and lung density assessed by magnetic resonance imaging (2) in healthy subjects and subjects with respiratory disease (16). The $r_{\text{AiDA}}$ values derived from the technique are root mean square (RMS) averages of the various distances in the entire acinar airspace complex that are obtained from analysis of the recovery ($R$) after a series of breath holds. A secondary parameter conceptually corresponding to particle recovery after a 0-s breath hold ($R_0$) can also be derived from AiDA measurements and is hypothesized to hold additional information about the small conducting airways (2, 23). However, interpretation of the data is still unclear and needs to be investigated further. Experimental data with AiDA for different regions of the respiratory tract is also lacking.

The objective of this study was to evaluate AiDA as a technique to assess $r_{\text{AiDA}}$ at different volumetric sample depths in the lungs for healthy adults. We also investigate the relationships between obtained $r_{\text{AiDA}}$ values, $R_0$, and established morphological lung models based on histological data (9, 43, 44, 46).

**METHODS**

Subjects and experimental design. The study was performed on 19 healthy subjects, 6 women and 13 men, aged 17–67 yr, recruited locally. All subjects underwent conventional lung function tests, according to current recommendations for diagnosis of respiratory disease (32). The subjects performed a series of, in total, 48 AiDA lung deposition measurements of nanoparticles at different breath holding times (5–10, 15, and 20 s) and varied exhaled volumetric sample depths [700, 1,000, 1,500, 2,000, and 2,500 ml and 1 individually adjusted measurement close to total lung capacity (TLC)]. To test the reproducibility of the measurements, and to ensure that the AiDA setup performed consistently, duplicate measurements were performed on 3 subjects with 15, 17, and 18 mo between the measurements. For one of the subjects, triplicate measurements were made. This also provided information about the reproducibility of the technique. The study was approved by the regional ethical review board of Lund and was performed in accordance with the Declaration of Helsinki. An informed written consent was obtained from all subjects.

Pulmonary function tests. The lung function tests included postbronchodilatation measurement of forced expiratory volume in 1 s (FEV1) and vital capacity (VC), as well as diffusing capacity for carbon monoxide (Jaeger MasterScreen PFT, IntraMedic, Sollentuna, Sweden) and determination of static lung volumes by body plethysmography (Jaeger MasterScreen PFT, IntraMedic). Lung function variables were reported both as absolute values and as normalized percentage of predicted values (32).

Particle deposition measurements. The instrumentation used for the particle deposition measurements has been described in detail elsewhere (17). A schematic illustration of the AiDA instrument is shown in Fig. 1.

A test aerosol was produced by aerosolizing polystyrene latex nanospheres (PSL) (Polymer Microsphere Suspension, Microgenics Corp, Fremont CA) with an electrospray aerosol generator (Model 3480, TSI Inc, Shoreview, MN). The produced aerosol was size controlled by a differential mobility analyzer (count mean diameter (CMD) = 51 nm, geometric standard deviation (GSD) = 1.14; DMA, Model 3071, TSI GmbH, Aachen, Germany) and diluted with particle free air to a concentration of 5,600 ± 1,600 particles/cm³ (mean ± SD). The size distribution of the test aerosol is shown in Fig. 2.

The test aerosol was continuously produced and led into a semi-flexible aerosol reservoir with an exhaust in a flow-through design that ensured a steady supply of fresh test aerosol during the measurements. A high-speed, computer-controlled valve administered test aerosol or particle-free air to the subjects through a mouthpiece connected to a pneumotachograph flow meter (as used in MasterScreen PFT, Viassys GmbH - Erich Jaeger, Hoechberg, Germany). The high-speed valve was connected to a sample collector, enabling sampling from well-defined exhaled volumes corresponding to volumetric lung sample depths. The particle concentration was monitored in the aerosol reservoir, and in the exhaled samples by a condensation particle counter (CPC; BCPC, Model a20, Airmodus Ltd., Helsinki, Finland). The aerosol was dried before it entered the CPC. The instrument was constructed according to the design criteria described by Löndahl et al. (24) and characterized for particle losses (17).

A measurement of particle recovery started with a period of tidal breathing with particle-free air during which the subject got comfortable with the instrument and ventilated the lungs. After a short time period (<30 s), the subject performed an exhalation to residual volume (RV), followed by a deep inhalation to TLC. When the inhalation started, the altered flow direction detected by the flow meter automatically triggered the high-speed valve to switch, so that the subject inhaled the test aerosol. After full inhalation, all valves were automatically shut, and the subject held his/her breath for a determined period (5–20 s). Thereafter, the valve opened toward the sample collector, and the subject exhaled to a predetermined volumetric sample depth before the valve was turned and the rest of the breath was discarded to exhaust. The breathing flow was recorded at 100 Hz by the pneumotachograph (Fig. 3A), and simultaneously the signal from the particle detector was recorded at 1 Hz (Fig. 3B). The particle detector measures from the aerosol reservoir until end of

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**Fig. 1.** The Airspace Dimension Assessment (AiDA) aerosol deposition measurement system and its subsystems: A: aerosol generation and conditioning system consists of an electrospray aerosol generator, a differential mobility analyzer (DMA) for particle size control, and a supply of particle-free dilution air. B: inhalation and sampling system consists of a flow meter and a computer-controlled, four-way valve that controls the flow of test aerosol to the subject and samples of exhaled aerosol to the sample collector. C: aerosol analysis system determines particle number concentration of the inhaled test aerosol and the exhaled samples by means of a condensation particle counter (CPC).

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**References**

(9, 43, 44, 46).
exhalation and thereafter from the sample collector (Fig. 1) (17). A volume of ~500 ml of the exhaled sample was analyzed by the CPC, starting from the top of the sample collector (the last exhaled sample volume) during 30 s at a flow rate of ~1 l/min.

The particle recovery, \( R \), was determined from the exhaled particle concentration divided by the inhaled particle concentration (8–10 s average before inhalation) and corrected for particle losses in the instrument caused by individual breathing flow rates as described elsewhere (17). The respiratory flow rates have previously been shown to have minimal influence on the measurements (17) and were thus not predetermined. The residence time in the lungs was defined as the time between midpoint time of inhalation and the midpoint time of exhalation in similarity with the standard for measurement of diffusing capacity for carbon monoxide (26).

Data analysis. The particle recovery data were used to calculate \( R_{\text{AIDA}} \) according to the procedure described by Löndahl et al. (23), using a theoretical model originally derived by Goldberg (12). The relation between particle recovery and residence time can be analytically solved for a lung geometry approximated by infinitely long and equally sized circular tubes or spheres. An exponential curve was fitted to the particle recovery as function of residence time for the aerosol in the lungs, \( R(t) \):

\[
R(t) = R_0 \cdot e^{\lambda t}
\]

\( R_0 \) is the extrapolated recovery at \( t = 0 \), and thus corresponds to an assumed recovery after a 0-s breath hold. \( \lambda \) is the decay constant for the nanoparticles. \( R_0 \) can conceptually be understood as an extrapolation to the expected particle recovery after inhalation and exhalation with no respiratory pause, although the theoretical background is somewhat more complex (12). \( R_0 \) is hypothesized to contain information about particle losses during the dynamic part of the breathing maneuver (i.e., during inhalation and exhalation).

The particle half-life time in the lung, \( \tau_{\text{DIA}} \), is directly related to the decay constant \( \tau_{\text{DIA}} = \ln(2)/\lambda \) and used to derive a characteristic \( R_{\text{AIDA}} \) corresponding to a hypothetical radius of the airspace where the aerosol has resided. \( R_{\text{AIDA}} \) at different volumetric sample depths, \( V \), are calculated, as described by Löndahl et al. (23), as:

\[
R_{\text{AIDA}(V)} = 2.89 \sqrt{D\tau_{\text{DIA}}(V)}
\]

where \( D \) is the particle size dependent diffusion coefficient.

Each exhaled sample generated information of \( R_{\text{AIDA}} \) and \( R_0 \) over a volume range of around 500 ml in the lungs. To provide a convenient metric for further statistical analysis, an average of the five lowest recorded \( R_{\text{AIDA}} \) at each volumetric sample depth were used. This approach was considered to provide the most accurate representation as the lowest values are least affected by foreseeable measurement artifacts such as residues of particles from the aerosol reservoir in the detector after valve switch. The averaged values were denoted \( R_{\text{AIDA}(700)} \) and \( R_{\text{AIDA}(2,500)} \). It should be noted that the volume notation is nominal and that the volumes were corrected for dead volume in the instrument, calibration of the pneumotachograph, and flow rate of the particle detector, which was measured at each measurement occasion. In the same way, the averages for the \( R_0 \) parameter were formed for the same data points, denoted \( R_{0(700)} \) and \( R_{0(2,500)} \).

Statistical analysis was made with IBM SPSS Statistics 23 (Release 23.0.0.0, 2015). Correlations were investigated with Spearman’s rank test and significances considered were those with \( P < 0.05 \); group differences were investigated with the Mann-Whitney U-test.

RESULTS

Subject demographics and lung function tests. Demographics and average lung function parameters for the subjects are provided in Table 1. All subjects had normal lung function and showed no signs of respiratory disease or abnormalities.

Effective airspace radius and zero second recovery. Average \( R_{\text{AIDA}} \) at different volumetric sample depths for the whole group are shown in Fig. 4 and are also given in Table 2. As seen, \( R_{\text{AIDA}} \) reach a uniform value at volumetric
sample depths ≥ 1,000 ml, where it is on average 276 ± 25 μm for the group. This is interpreted as representing a value for homogeneous acinar lung tissue. The intersubject variability, given as the coefficient of variation, was close to 10% (7.4%–11.1%) for the examined volumetric sample depths with a minimum for \( r_{AIDA(\text{Max})} \). The \( R_0 \) parameter (Fig. 5) showed higher intersubject variability with coefficient of variation ranging from 33% at \( R_{0(700)} \) to 87.5% at \( R_{0(\text{Max})} \). Thus, the intersubject variability is higher for particle losses during the dynamic part of the breathing protocol than for the static breath-hold period, which is expected to reflect diffusional deposition in the gas exchange region. \( R_0 \) did not correlate with flow rate during inhalation or exhalation. No group differences could be found between male and female subjects, with exception for \( r_{AIDA(700)} \) (\( P < 0.021 \)), which may be related to the difference in body size and thus relative volume of anatomical dead space. One subject showed a somewhat atypical pattern for \( r_{AIDA} \), with values 20%–27% above average for intermediate volumetric sample depths (1,000–2,500 ml) but average at \( r_{AIDA(\text{Max})} \). \( R_0 \) values were also atypical for some of the measurements on this individual. For one subject, the exhaled sample volumes exceeded the inhaled test aerosol volume and thus data had to be discarded for that measurement.

Figure 5 shows the average \( R_0 \) for all subjects. As seen, \( R_0 \) decreases with volumetric sample depth in the lung. The average values of \( r_{AIDA} \) are also given in Table 2.

Generally, \( r_{AIDA} \) values measured at different volumetric sample depths on the same subject correlated with each other (\( P < 0.0000001–0.0053 \), Spearman’s \( \rho = 0.51–0.89 \)) with exception of \( r_{AIDA(\text{Max})} \), where only a weak correlation to \( r_{AIDA(700)} \) was found (\( P = 0.037 \), Spearman’s \( \rho = 0.51 \)). A similar pattern was observed for the averaged \( R_0 \) values, but without exceptions for any volumetric sample depths (\( P < 0.0000001–0.012 \), Spearman’s \( \rho = 0.58–0.95 \)).

### Table 1. Descriptive demographic data and lung function parameters for all subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Range (Min–Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>34</td>
<td>14</td>
<td>17–67</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177</td>
<td>8</td>
<td>151–186</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78</td>
<td>15</td>
<td>54–107</td>
</tr>
<tr>
<td>TLC, liters</td>
<td>7.02</td>
<td>1.08</td>
<td>4.66–8.91</td>
</tr>
<tr>
<td>TLC%Pred, %</td>
<td>103</td>
<td>12</td>
<td>84–121</td>
</tr>
<tr>
<td>RV, liters</td>
<td>1.72</td>
<td>0.49</td>
<td>1.20–2.81</td>
</tr>
<tr>
<td>RV%Pred</td>
<td>118</td>
<td>40</td>
<td>80–252</td>
</tr>
<tr>
<td>FRC, liters</td>
<td>3.60</td>
<td>0.84</td>
<td>2.41–5.19</td>
</tr>
<tr>
<td>FRC%Pred, %</td>
<td>103</td>
<td>17</td>
<td>69–134</td>
</tr>
<tr>
<td>VC, liters</td>
<td>5.22</td>
<td>0.76</td>
<td>3.50–6.79</td>
</tr>
<tr>
<td>VC%Pred, %</td>
<td>100</td>
<td>11</td>
<td>80–121</td>
</tr>
<tr>
<td>FEV1, liters</td>
<td>4.16</td>
<td>0.58</td>
<td>3.09–5.29</td>
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<td>FEV1/VC, %</td>
<td>101</td>
<td>11</td>
<td>82–118</td>
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<tr>
<td>FEV1/VCO2, %</td>
<td>80.3</td>
<td>5.7</td>
<td>72.0–93.7</td>
</tr>
<tr>
<td>FEV1/VCO2, %</td>
<td>101</td>
<td>6</td>
<td>92–116</td>
</tr>
<tr>
<td>DLCO, mmol·min⁻¹·kPa⁻¹</td>
<td>10.13</td>
<td>1.80</td>
<td>6.16–13.55</td>
</tr>
<tr>
<td>DLCO%Pred, %</td>
<td>102</td>
<td>13</td>
<td>70–122</td>
</tr>
<tr>
<td>KCO, mmol·min⁻¹·kPa⁻¹</td>
<td>1.59</td>
<td>0.20</td>
<td>1.31–2.20</td>
</tr>
<tr>
<td>KCO%Pred, %</td>
<td>98</td>
<td>17</td>
<td>70–133</td>
</tr>
</tbody>
</table>

\( n = 19 \) (6 women and 13 men). \%Pred, percentage of predicted; DLCO, diffusing capacity for carbon monoxide; FEV1, forced expiratory volume in 1 s; FRC, functional residual capacity; KCO, carbon monoxide transfer coefficient; Max, maximum; Min, minimum; RV, residual volume; TLC, total lung capacity; VC, vital capacity. *\( n = 18 \), data missing for one subject.

\( \rho = 0.48 \), but comparison between the younger and older subjects did not show a significant group difference for this parameter (\( P = 0.09 \)). Correlations were also found between \( r_{AIDA} \) and age (\( P < 0.014–0.04 \), Spearman’s \( \rho = 0.47–0.57 \)). Weight (\( P < 0.04 \), Spearman’s \( \rho = 0.50 \)), and clinical lung function tests (TLC, RV, functional residual capacity, FEV1/VC), but not at all volumetric sample depths. When the pulmonary function tests were expressed as percentage of predicted, the correlations were fewer and weaker. Correlation was also found between \( R_0 \) and carbon monoxide transfer coefficient (KCO) at all volumetric sample depths (\( P < 0.0046–0.048 \), Spearman’s \( \rho = 0.46 \) to \( -0.62 \)), but not to any other anthropometry or lung function tests.

**Aida and subject characteristics.** Correlations were found between the AIDA measurements, anthropometry (most notably, age), and lung function tests (Table 3), but no correlation was found between \( r_{AIDA} \) and \( R_0 \), which indicates that \( r_{AIDA} \) and \( R_0 \) may give information about independent lung properties. Correlations were found between \( r_{AIDA} \) and age for volumetric sample depths between 1,000–2,500 ml (with \( P < 0.007 \), Spearman’s \( \rho = 0.60 \) at 2,500 ml). Comparing younger subjects (<30 yr, \( n = 9 \)) with older subjects (>30 yr, \( n = 10 \)) showed a significant group difference (\( P = 0.014 \)) with an average \( r_{AIDA(2,500)} \) of 256 ± 13 μm for the younger group and 284 ± 29 μm for the older group, respectively. Correlations were also found between \( R_0 \) and age (\( P < 0.037 \), Spearman’s \( \rho = 0.48 \)), but comparison between the younger and older subjects did not show a significant group difference for this parameter (\( P = 0.09 \)). Correlations were also found between \( R_0 \) and height (\( P < 0.014–0.04 \), Spearman’s \( \rho = 0.47–0.57 \)), weight (\( P < 0.04 \), Spearman’s \( \rho = 0.50 \)), and clinical lung function tests (TLC, RV, functional residual capacity, FEV1/VC), but not at all volumetric sample depths. When the pulmonary function tests were expressed as percentage of predicted, the correlations were fewer and weaker. Correlation was also found between \( R_0 \) and carbon monoxide transfer coefficient (KCO) at all volumetric sample depths (\( P < 0.0046–0.048 \), Spearman’s \( \rho = 0.46 \) to \( -0.62 \)), but not to any other anthropometry or lung function tests.
In addition to these significant correlations, there were several indications (0.05 < \( P < 0.1 \)) that more relations may be present between the AiDA variables and lung function tests, although not substantiated by the data in this study consisting of a limited, homogenous group of healthy subjects. Importantly, there were no correlations between \( r_{\text{AiDA}} \) and \( R_0 \), with exception for a correlation between \( r_{\text{AiDA}}(\text{Max}) \) and \( R_0 \) (\( P < 0.0045 \), Spearman’s \( \rho = -0.64 \)). This suggests that the two variables are independent and contain information about different lung properties. Correlations with some anthropometric and lung function tests for two volumetric sample depths (700 ml and 2,000 ml) are shown in Table 3.

**Repeatability.** As demonstrated in Figs. 6 and 7, repeated measurements on 3 subjects (S01: male, 37 yr; S02: male, 38 yr; and S03: male, 64 yr) yield almost identical results for \( r_{\text{AiDA}} \), even when measured at long (18 mo for S01, 17 mo for S02, and 15 mo for S03) intervals. Some differences at low sample depths were observed, but these are most likely related to the precision of the volume measurement (see Fig. 7B). No significant change in \( r_{\text{AiDA}} \) was observed over this time interval (on average <2.4% or <17 \( \mu m \) for volumetric sample depths between 1,000 and 2,500 ml for the 3 subjects). Figure 7 shows triplicate measurements on S01, at the beginning of the study (Start), after 10 mo (Mid), and after 18 mo (End). The increase in \( r_{\text{AiDA}} \) at the maximum volumetric sample depths (Fig. 7A) is interpreted as an effect of low particle concentrations close to the CPC detection limit.

**DISCUSSION**

In this study, we used AiDA (17, 23) to assess \( r_{\text{AiDA}} \) and \( R_0 \) at volumetric lung sample depth from \(-200\) to 5,000 ml in 19 healthy subjects. Both \( r_{\text{AiDA}} \) and \( R_0 \) correlated with anthropometry and lung function parameters, but importantly, \( r_{\text{AiDA}} \) and \( R_0 \) did not correlate with each other with exception for a weak correlation between \( r_{\text{AiDA}}(\text{Max}) \) and \( R_0(\text{Max}) \). This indicates that they reflect independent lung properties.

\( r_{\text{AiDA}} \) should not be understood as an anatomical measurement of alveolar size or bronchiole caliber at a specific airway generation, but rather as representative effective RMS radii to surfaces in the lungs at a specific volumetric sample depth that include several generations of the distal lungs.

The \( r_{\text{AiDA}} \) values were larger than typical sizes for lung models at the same volumetric sample depths, but this is expected as the \( r_{\text{AiDA}} \) values are RMS distances in the respiratory zone at full lung inflation. Distances from histology are arithmetic means, which are lower than RMS distances for the same geometry. The average \( r_{\text{AiDA}} \) value at 1,000–2,500 ml volumetric sample depth was \( 276 \pm 25 \mu m \) (equal to \( 552 \pm 50 \mu m \) diameter), which can be compared with typical dimensions given by lung models for the respiratory zone, i.e., \( 540 \mu m \) (diameter) for airway generation 17 (46). This indicates that the \( r_{\text{AiDA}} \) signal from the examined volumetric sample depths correspond to a signal that is mostly influenced by features of the lungs around and distal to airway generation 17. The values given by most lung models are scaled to a relaxed lung (15), whereas \( r_{\text{AiDA}} \) is for full lung inflation, but this has a minor influence on measured airspace radii, as this scales approximately to the cube root of volume.

The general pattern of the \( r_{\text{AiDA}} \) values was consistent for all participants (Figs. 4 and 5, Table 2), although significant intersubject variations were observed. The intersubject variability for \( R_0 \) was larger than for \( r_{\text{AiDA}} \), which indicates larger differences for the particle losses during the dynamic part of the breathing maneuver than during the static breath hold. As seen in Figs. 6 and 7, the repeatability for \( R_0 \) was also lower compared with \( r_{\text{AiDA}} \), which further increases the variability. The larger intersubject variability for \( R_0 \) could be suspected to originate from the individual breathing patterns, but it has previously been shown that particle deposition is primarily determined by the residence time in the lungs and not strongly by altered breathing patterns (17).


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Table 3. Correlations between \( r_{\text{AiDA}} \) and \( R_0 \) at 700 ml and 2,000 ml

<table>
<thead>
<tr>
<th>( r_{\text{AiDA}}(700) )</th>
<th>( r_{\text{AiDA}}(2,000) )</th>
<th>( R_0(700) )</th>
<th>( R_0(2,000) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.464</td>
<td>0.600*</td>
<td>0.321</td>
</tr>
<tr>
<td>Height</td>
<td>0.570*</td>
<td>0.351</td>
<td>-0.306</td>
</tr>
<tr>
<td>Weight</td>
<td>0.485*</td>
<td>0.213</td>
<td>-0.231</td>
</tr>
<tr>
<td>TLC</td>
<td>0.472*</td>
<td>0.468*</td>
<td>0.137</td>
</tr>
<tr>
<td>RV</td>
<td>0.430</td>
<td>0.404</td>
<td>0.052</td>
</tr>
<tr>
<td>FRC</td>
<td>0.389</td>
<td>0.458*</td>
<td>0.199</td>
</tr>
<tr>
<td>FEV1/VC</td>
<td>-0.290</td>
<td>-0.491*</td>
<td>-0.086</td>
</tr>
<tr>
<td>( K_{\text{CO}} )</td>
<td>-0.135</td>
<td>-0.304</td>
<td>-0.569*</td>
</tr>
</tbody>
</table>

\( r_{\text{AiDA}} \), Airspace Dimension Assessment; \( \text{FEV}_1 \), forced expiratory volume in 1 s; FRC, functional residual capacity; \( K_{\text{CO}} \), carbon monoxide transfer coefficient; \( \rho \), Spearman’s rank correlation coefficient; \( R_0 \), recovery after 0-s breath hold; RV, residual volume; TLC, total lung capacity; VC, vital capacity. *Significant at \(< 0.05 \) level. **Significant at \(< 0.01 \) level.

more likely that the intersubject variability reflected variations in individual lung morphology. Supporting evidence for this interpretation has recently been published by the authors in a study comparing lung deposition of nanoparticles performed with the same set-up as used in this study (16).

The results from this study imply that AiDA is most consistent at volumetric sample depths between 1,000–2,500 ml for most subjects (Figs. 4 and 7). Breath-holding times between 5 and 10 s generally give exhaled particle levels that are most suited for determining \( r_{\text{AiDA}} \). The largest measurement uncertainty was observed for the shallowest and the deepest volumetric sample depths. For the shallowest volumetric sample depths, the measurement uncertainty was interpreted as mainly related to accuracy of the volume determination combined with rapidly decreasing \( r_{\text{AiDA}} \). Even a small measurement error of the volumetric sample depths from this transitional region results in a significant measurement uncertainty. The uncertainty at the deepest volumetric sample depths can be explained by the low concentrations of exhaled particles, which were close to the detection limit of the particle detector. Additional causes of uncertainty, observed for some participants, were sample depths close to TLC, resulting in strained exhalation and possibly effects of airway closures and uneven exhalation. For one subject, the exhaled sample volumes exceeded the inhaled test aerosol volume, and thus data had to be discarded.

The AiDA technique was found to be reproducible regarding both \( r_{\text{AiDA}} \) and \( R_0 \) (Figs. 6 and 7). This has also been shown previously by measuring lung deposition of nanoparticles with

![Fig. 6](image.png)  
**Fig. 6.** A: comparison of 2 measurements of 0-s recovery (\( R_0 \)) for 3 subjects at the beginning of the study and at a measurement performed several months later (18.5 mo for S01, 17 mo for S02, and 15 mo for S03). B: comparison of effective airspace radii (\( r_{\text{AiDA}} \)) for the same measurements.

![Fig. 7](image.png)  
**Fig. 7.** A: effective airspace radii (\( r_{\text{AiDA}} \)) for one subject (male, 37 yr) at the beginning of the study, at a midpoint (10 mo), and at a measurement performed 18.5 mo after the first measurement. \( r_{\text{AiDA}}(700) \)-\( r_{\text{AiDA}}(2,000) \) values are displayed with error bars indicating \( \pm 1 \) SD but are not visible as they are on average within \( \pm 5 \) \( \mu \text{m} \). B: \( R_0(700) \)-\( R_0(2,000) \) values for the same measurements. \( R_0 \), 0-s recovery.
In this study, with a group difference for
further estimated an expected slower EAD increase after the
Nevertheless, the shallowest volumetric sample depth deviated from
subjects (23–80 yr) and found that EADs increased from ~184
same authors examined growth of the small airways and alveoli
bronchioles. They could identify two distinct regions where
parameters (TLC, RV, functional residual capacity, FEV1/VC) at
the observation is an effect of this anatomical transition. There
models for the relationship between EADs and particle recovery
from the respiratory tract of micrometer sized, monodisperse particles, e.g., different versions of ADAM (3, 10, 13, 21, 29–31). ADAM has been shown to be able to give EADs closely related to anatomical dimensions, validated on mechanically ventilated model animals, excised dog lungs (36), excised donkey lungs (40), and excised human lungs (28), as well as for physical models with well-defined geometrical dimensions (7). In particular, two studies have used ADAM to study EADs for relatively large healthy populations. Brand et al. (6) measured EADs on 79 healthy subjects in a wide age span (20–80 yr) to establish normal reference data. They found that EADs agreed reasonably well with the expected results from lung models [Weibel (43) and Yeh & Schum (47) lung models], but gave considerable larger EADs for shallow sample depths (<15% TLC) and slightly lower EADs for deeper sample depths. On average, the EADs measured by Brand et al. (6) were around 260 μm for sample depths >15% TLC compared to ~400 μm for the Weibel model and 380 μm for the Yeh and Schum model. The authors explained this discrepancy with the fact that ADAM gives an average value for conducting airways and alveoli, whereas the lung models estimate the bronchioles as tubes. Similar trends were found by Zeman et al. (49) for 54 healthy subjects (18–70 yr), who estimated an EADmin (corresponding to the deepest valid sample depth in their study) to 273 ± 60 μm.

Two theoretical models for ADAM are described in the literature: the tube model (14) and the cord-length model (35). Similarly to the model evaluated in this study, the tube model (14) assumes that inhaled particles deposit in a system of identical but randomly oriented tubes, but exclusively by sedimentation. The cord-length model (35) also assumes that particles deposit by sedimentation but in a geometry described by a frequency distribution of path lengths, defined by the lung anatomy, where the linear mean intercept, Lm, is the defining parameter. Even though the models simplify the lung anatomy in different ways, they reach similar relationships between particle recovery and EADs (3). The relationship between airspace dimension and particle half-life time in the lung for the two models (analogous to the model used in this study) can be estimated as

\[ r_{\text{tube}} = 0.92v_r l_{\text{tg}} \tag{3} \]

\[ r_{\text{chord}} = 0.90v_r l_{\text{tg}} \tag{4} \]

where \( r \) is the derived airspace radius for the different models, \( v_r \) is the terminal settling velocity for the aerosol particles, \( l_{\text{tg}} \) is the particle half-life time, and the constants are model-specific parameters. This can be compared with the AiDA model, Eq. 2, based on diffusional deposition in a lung anatomy simplified in a similar way as the tube model (23). The relationship between \( t_{\text{tg}} \) and radius in the ADAM models (Eqs. 3 and 4) is linear, whereas the particle half-life time in the
AiDA model is proportional to the airspace radius squared, thus it is an RMS dimension (23). This has both theoretical and practical implications when comparing the techniques.

The differences in theoretical models between AiDA and ADAM have important implications for interpretation of data. First, a minority of larger airspaces in the lungs increases $r_{AIDA}$ substantially since these are RMS values, whereas they have a minor effect on average EADs derived by ADAM. The sensitivity to a small number of enlarged airspaces in AiDA is assumed to be a significant advantage in diagnostic applications for emphysema, which is one of the main suggested applications of the AiDA technique. Second, nanoparticles are expected to penetrate and spread more evenly by diffusion in the lung compared with micron-sized particles (23), especially that may cause unpredictable and locally high particle deposition.

In summary, the AiDA technique as applied in this study provides a robust, noninvasive in vivo technique to assess $r_{AIDA}$ in the distal lung. The derived $r_{AIDA}$ values should not be understood as a physical radius of an average airspace, but rather as a measurement of a functional average diffusion distance to a surface in the lung, weighted toward larger airspaces, but not with each other, which indicates that they convey independent information. The method may be of value for future lung diagnostic applications; however, the clinical value remains to be studied on larger populations including subjects with respiratory disease. The data presented in this study form the basis for an optimized measurement procedure in future studies addressing these questions.

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DISCLOSURES

J. Löndahl and P. Wollmer have a patent pending for the AiDA technology, a device and method for pulmonary function measurement, application number PCT/EP2013/073977. J. Jakobsson reports no conflicts of interest of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

J.J., P.W., and J.L. conceived and designed research; J.J. performed experiments; J.J., P.W., and J.L. analyzed data; J.J., P.W., and J.L. interpreted results of experiments; J.J. and J.L. prepared figures; J.J. drafted manuscript; J.J., P.W., and J.L. edited and revised manuscript; J.J., P.W., and J.L. approved final version of manuscript.

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